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Salicylic Acid Signaling: Biosynthesis, Metabolism, and Crosstalk with Jasmonic Acid

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Salicylic acid (SA) signaling plays an important role in local and systemic acquired resistance. Expression and activity of pathogenesis-related proteins are stimulated by the accumulation of SA, conferring resistance to pathogens. SA can be synthesized via the phenylpropanoid route or the isochorismate pathway and metabolized to form SA-glucoside and SA glucose-ester through glucosylation, and methyl salicylate through methylation. This summary focuses on genes involved in SA biosynthesis, metabolism, and signaling. SA and jasmonic acid (JA) crosstalk has an important role in regulating induced defense against pathogens by exerting antagonistic effects. Therefore, results on crosstalk between SA and JA are also shortly reviewed. Further investigation on the molecular aspect of SA and JA antagonism, elucidating how these pathways are linked to each other, and how they resolve the complexity of host-pathogen interaction will provide a better understanding on SA signaling and plant defense.

Key words: salicylic acid, salicylic acid metabolism, salicylic acid synthesis, systemic acquired resistance

Plant defense is an important mechanism that combats different types of pathogens. A distinct signal transduction pathway, referred to as systemic acquired resistance (SAR), plays a significant role in the ability of plants to defend themselves against pathogens [Ryals *et al.*, 1996]. SAR activation results in a broad-spectrum, long lasting immunity in non-infected tissues [Hunt and Ryals, 1996]. It is associated with the expression of genes called SAR genes. In tobacco and Arabidopsis, SAR marker genes pathogenesis-related (*PR*)-*1a* and *PR1* are the most abundant and tightly correlated with the plant defense responses against pathogen attack [Ward *et al.*, 1991]. The accumulation of salicylic acid (SA), an endogenous signaling molecule, is an essential process in SAR. SA accumulation is correlated with the induction of PR proteins [Loake and Grant, 2007]. Mutant analysis, involving constitutively activated mutants and SAR-compromised mutants, was conducted to determine the genetic mechanism in SAR signal transduction pathway [Ryals *et al.*, 1996]. In Arabidopsis, for example, *lesions simulating disease* mutants (*lsd1* to *lsd7*) [Dietrich *et al.*, 1994], *accelerated cell death2* (*acd2*) [Greenberg *et al.*, 1994], *accelerated cell death6* (*acd6*) [Lu *et al*., 2003], and *aberrant growth and death2* (*agd2*) mutants [Rate and Greenberg, 2001] have been identified. These mutants exhibit spontaneous lesion formation phenotype, elevated SAR gene expression, increased SA levels, and resistance to pathogens. In SAR-compromised mutants, defective SA accumulation, absence of PR gene activation, and susceptibility to pathogen were observed. The mutants include *NahG* (bacterial salicylate hydroxylase gene)-overexpressing transgenic plants, which facilitates the conversion of SA to catechol [Gaffney *et al.*, 1993], *noninducible immunity* (*nim1*) [Delaney *et al.*, 1995], *nonexpressor of PR gene1* (*npr1*) [Cao *et al.*, 1994], *phytoalexin deficient4* (*pad4*) [Zhou *et al.*, 1998], and *salicylic acid induction-deficient2* (*sid2*) mutants [Wildermuth *et al.*, 2001].

Much progress has been made in elucidating the mechanisms involve in SAR and SA signaling pathway [reviews by Ryals *et al.*, 1996; Melchers and Stuiver, 2000; Pieterse *et al.*, 2001; Punja, 2001; Shah, 2003; Durrant and Dong, 2004, Loake and Grant, 2007]. Thus, in regard to the importance of SA in plant defense

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response, this review focuses on recent studies in the molecular characterization and regulation of genes involved in SA biosynthesis and metabolism. It also discusses the antagonism between two different pathways: SA and jasmonic acid (JA) signalings.

Biosynthetic Pathway of SA

SA is synthesized through two different pathways: the phenylpropanoid and the isochorismate pathways (Fig. 1). Biosynthesis of SA was initially studied biochemically in tobacco leaves, leading to the discovery of the cytoplasmic phenylpropanoid pathway. It begins with the conversion of phenylalanine to *trans*-cinnamic acid (*t*-CA), which is catalyzed by phenylalanine ammonia-lyase (PAL); *t*-CA is then converted to benzoic acid, and SA is derived from benzoic acid via hydroxylation and catalyzed by benzoic acid 2-hydroxylase (BA2H) [Yalpani *et al.*, 1993; Ryals *et al.*, 1996]. This is supported by the experiment in transgenic tobacco, wherein suppression of one PAL gene resulted in decreased SA accumulation in response to tobacco mosaic virus (TMV) inoculation [Pallas *et al.*, 1996]. More recent studies in Arabidopsis indicate that SA can also be synthesized from chorismate in the chloroplast [reviews by Shah, 2003; Durrant and Dong, 2004]. Conversion of chorismate to isochorismate is facilitated by the enzyme isochorismate synthase (ICS). Isochorismate is then converted to SA by isochorismate pyruvate lyase (IPL).

Study of a *salicylic acid-induction-deficient2* (*SID2*) gene encoding ICS1 showed that *ICS1* is induced locally and systemically during pathogen infection, and the SA level in *sid2* mutants was about 5-10% of the wild-type [Wildermuth *et al*., 2001], suggesting that SA synthesis by ICS1 is required for SAR.

Zhang *et al*. [2010] reported that two members of a plant-specific family of transcription factors, SAR Deficient1 (SARD1) and CBP60g, regulate the induction of *ICS1* and SA synthesis. A highly conserved central region of the two proteins facilitates binding to the DNA. Results showed that SAR was compromised in *SARD1* knockout plants, whereas enhanced resistance was observed in *SARD1*-overexpressing plants. Moreover, they showed that SARD1 is targeted to the *ICS1* promoter after pathogen infection.

Expression patterns of *PAL* and *ICS* and their enzymatic activities were examined to understand the synthesis of SA in probenazole (PBZ)-treated Arabidopsis [Yu *et al.*, 2010]. PBZ is a distinct SAR-inducer [Yoshioka *et al*., 2001]. Results showed the expression level of *ICS1* and its enzymatic activity were increased by PBZ treatment. Free and total SAs were also increased in

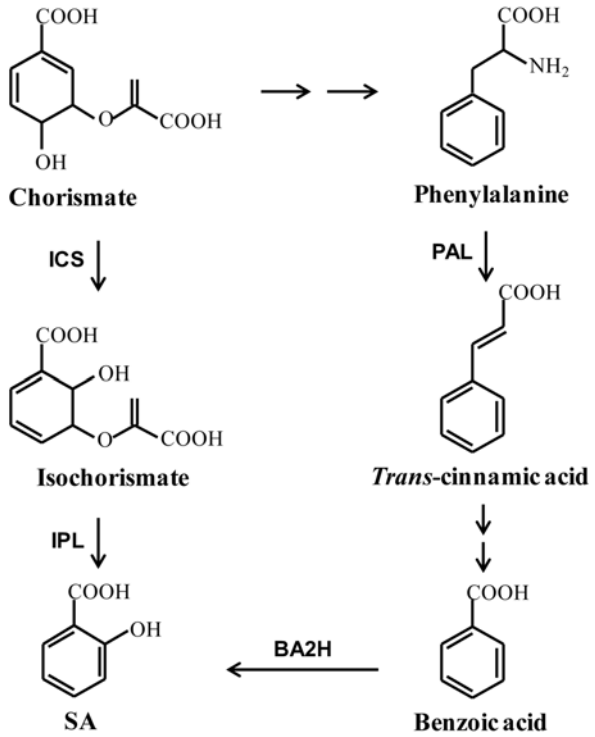

Fig. 1. Proposed pathways for SA biosynthesis [Shah, 2003; Yu *et al.*, 2010]. There are two pathways in SA synthesis, the phenylpropanoid pathway and the isochorismate pathway. In the phenylpropanoid pathway, occurring in the cytoplasm, phenylalanine is converted to *trans*-cinnamic acid (*t*-CA), catalyzed by PAL. *t*-CA is converted to benzoic acid and then to SA, catalyzed by BA2H. The isochorismate pathway, occurring in the chloroplast, involves the conversion of chorismate to isochorismate, catalyzed by ICS. Isochorismate is converted to SA by IPL.

PBZ-treated WT plants, but not in the *sid2-2* mutant. On the other hand, PAL expression and its enzymatic activity decreased in PBZ-treated WT plants. This shows that SA is mainly synthesized in the ICS-mediated pathway rather than the PAL-mediated pathway in PBZ-treated Arabidopsis.

Metabolic Pathway of SA and Its Conjugates

SA exists as free acid or conjugated products in plants [Lee *et al.*, 1995]. These conjugated products are formed through glucosylation and methylation (Fig. 2). Glucosylation can occur in either the hydroxyl or the carboxyl group to form SA glucoside (SAG; 2-*O*-β-D-glucoside), a major metabolite, and SA glucose-ester (SGE), a minor metabolite (Fig. 2). SA glucosyltransferase (SA GT) catalyzes the conversion of SA to SAG and SGE. Methylation of SA results in the formation of methyl salicylate (MeSA). It is synthesized by SA carboxyl methyltransferase (AtBSMT1), which is induced by either methyl jasmonate (MeJA) or a bacterial pathogen *Pseudomonas syringae* infection [Koo

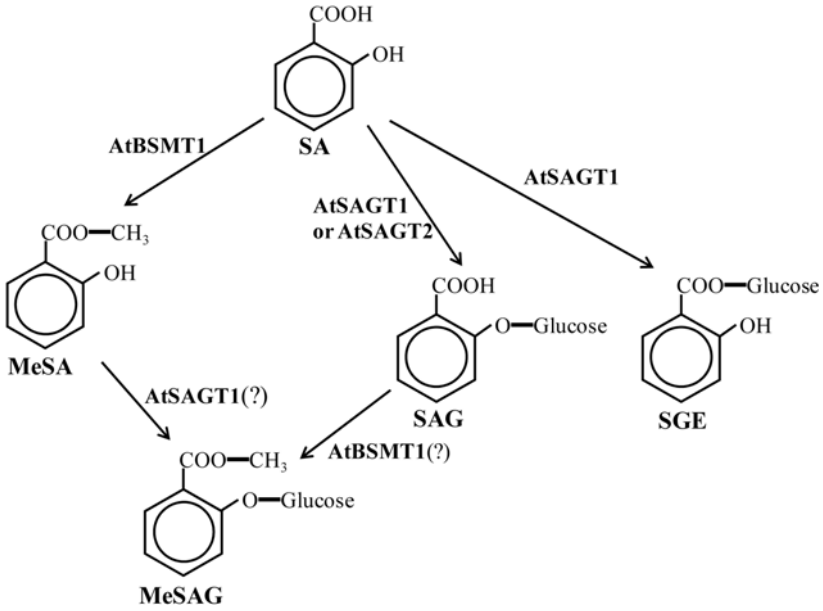

Fig. 2. SA and its metabolites [Song *et al.*, 2009]. SA metabolites include SAG (2-*O*-β-D-glucoside), SGE (SA glucose-ester), MeSA (methyl salicylate), and MeSAG (methyl salicylate 2-*O*-β-D-glucoside). SAG and SGE are catalyzed by AtSAGT1, whereas MeSA is catalyzed by AtBSMT1. MeSAG formed from SAG and MeSA may be synthesized by AtBSMT1 and AtSAGT1, respectively.

et al., 2007; Song *et al.*, 2008].

For the molecular characterization of SA GT, a pathogen-inducible gene, *Arabidopsis thaliana UDP-glucose:SA glucosyltransferase1* (*AtSAGT1*, formerly known as *AtSGT1*), encoding a protein with SA GT activity was isolated [Song, 2006]. AtSAGT1 was selected from six candidate genes annotated among the glucosyl transferases located in the Arabidopsis genome. At2g43820 gene product, having the highest homology with the tobacco SA GT, was assigned as AtSAGT1. The recombinant AtSAGT1 protein had significant activities with SA and BA, resulting in the synthesis of SAG and SGE. *AtSAGT1* was induced by pathogen inoculation and SA treatment. *AtSAGT1* transcript levels increased in *P. syringae*-infected and MeSA-treated leaves, suggesting that AtSAGT1 plays an important role in SA metabolism and plant defense response.

To determine the action of glucosyltransferases and its role in defense response, transgenic Arabidopsis plants overexpressing AtSAGT1 were analyzed [Song *et al.*, 2008]. Results showed that overexpression of *AtSAGT1* increased the levels of MeSA and MeSAG. However, susceptibility to *P. syringae* was also increased, thereby reducing the accumulation of free SA and its glucosylated forms, SAG and SGE.

For the analysis of SA carboxyl methyltransferase, *OsBSMT1*, an SA carboxyl methyltransferase gene from the rice genome, was transferred into Arabidopsis to generate a MeSA-overproducing transgenic plant [Koo *et al.*, 2007]. The recombinant OsBSMT1 protein showed carboxyl methyltransferase activity with SA and BA, and produced MeSA and MeBA, respectively. Overexpression of *OsBSMT1* increased the production of MeSA, which acts as an airborne signal to neighboring plants. However, the process of converting SA to MeSA resulted in the depletion of the active SA pool, thereby leading to susceptibility to *P. syringae* and a fungal pathogen *G. orontii*.

To further understand how AtSAGT1 and AtBSMT1 are regulated during disease response, transcript levels of *AtBSMT1* and *AtSAGT1* in plants with altered levels of SA and other defense components were assayed [Song *et al.*, 2009]. *AtSAGT1* expression was regulated partially by SA or NPR1, whereas the *AtBSMT1* expression was induced in SA-deficient mutants, demonstrating that low accumulation of SA caused more strong induction of *AtBSMT1* and other JA-responsive genes. This result was consistent with the previous study showing that SA elimination results in strong induction of JA signals through antagonistic effects [Koo *et al.*, 2007].

Crosstalk between SA and JA Signaling Pathways

SA and JA have fundamental roles in the regulation of induced plant defenses against pathogens. Cross-communication leads to the activation and fine tuning of defense responses [Durner *et al.*, 1997]. Jasmonate and its

metabolites, lipid derived compounds, are synthesized upon pathogen infection or insect attack. SA-mediated defenses are predominantly effective against biotrophic pathogens, such as *P. syringae*, whereas JA-mediated defenses are primarily effective against herbivorous insects and necrotrophic pathogens [Koornneef and Pieterse, 2008]. JA can be metabolized to methyl jasmonate (MeJA) via JA carboxyl methyltransferase (JMT) [Seo *et al.*, 2001].

SA and JA signalings cross talk at multiple regulatory points. Several studies showed that JA negatively regulates the expression of SA-responsive genes in Arabidopsis [Petersen *et al.*, 2000; Kachroo *et al.*, 2001]. Recently, Koo *et al.* [2007] strongly suggested that AtBSMT1 has a possible role in SA and JA interactions. They revealed that JA induces *AtBSMT1* expression, causing SA depletion by converting SA to MeSA, which may eventually contribute to an antagonistic effect on SA signaling.

Different regulatory proteins participate in the crosstalk between SA and JA. Koornneef and Pieterse [2008] discussed some of these regulators, which include the NPR1, a regulatory protein that is required for transduction of the SA signal; the WRKY transcription factor, specifically WRKY70, which regulates SA-mediated defenses while repressing the JA response, the glutaredoxin GRX480, which affects only a subset of the JA-responsive genes that are sensitive to SA-mediated suppression, and the MAP kinase4 (MPK4), a negative regulator of SA signaling and a positive regulator of JA signaling. JA-responsive regulatory genes include *plant defensin1.2* (*PDF1.2*), *lipoxygenase2* (*LOX2*), and *vegetative storage protein2* (*VSP2*).

In Arabidopsis, SA strongly antagonizes the JA signaling pathway, which results in the down-regulation of JA-responsive gene expression [Spoel *et al.*, 2003]. In a study by Spoel *et al.* [2003], SA-mediated suppression of JA-responsive genes *LOX2*, *VSP*, and *PDF1.2* was observed in wild type plants, whereas *npr1* mutants showed enhanced JA-responsive gene expression and increased levels of JA. This indicated importance of NPR1 in inhibition of JA-responsive gene expression by SA.

Leon-Reyes *et al.* [2010] reported that the antagonistic effect of SA on the expression of the JA-marker gene *PDF1.2* in different JA biosynthesis mutants showed down-regulation of the JA biosynthesis pathway is not essential for SA-mediated suppression of JA signaling. In mutant *aos/dde2*, where JA production is completely blocked, *PDF1.2* and *VSP2* were not expressed. However, exogenous application of MeJA rescued the JA-responsive phenotype in *aos/dde2*, and *PDF1.2* transcription induced by MeJA could still be antagonized by SA. This indicates that SA-mediated suppression of JA-responsive gene expression functions downstream of the JA biosynthesis.

Conclusion

SAR and its role in plant defense have been extensively studied over the recent years. Genetic engineering was very instrumental in understanding the signaling pathways and the molecular mechanisms involved. SA signaling pathway plays a vital role in SAR. SA accumulation results in the induction of PR proteins, which then leads to resistance to pathogens. Recent studies have characterized the molecular aspect of SA signaling, which include the gene expressions patterns in SA biosynthesis, metabolism, and crosstalk with JA [Durrant and Dong, 2004; Loake and Grant, 2007; Song *et al.*, 2008; Lu, 2009; Leon-Reyes *et al.*, 2010]. Crosstalk between SA and JA has an important role in regulating induced defense against pathogens by exerting antagonistic effects. However, there is a case wherein down-regulation of JA synthesis is not essential for SA-mediated suppression of JA. This deviating result from the main concept of mutually antagonistic relationship of SA and JA should be taken into consideration. Future prospect includes more advanced investigation on the molecular aspect of SA and JA antagonism to provide insight on what is really occurring within these two pathways. Different signaling pathways have been molecularly characterized and analyzed; however, the challenge to further understand how these pathways are linked to each other and eventually determining how, as a whole, they resolve the complexity of host-pathogen interaction still remain.

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